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HR Nuclear Magnetic Resonance Spectroscopy for authentication of olive oil quality

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Abstract

The present work is a preliminary study to settle the optimum experimental conditions and data processing for accomplishing the strategies established by the Action Plan for the EU olive oil sector.

The objectives of the work were: a) to monitor the evolution of extra virgin olive oil exposed to indirect solar light in transparent glass bottles during four months; b) to identify spectral differences between edible and lampant virgin olive oil by applying high resolution Nuclear Magnetic Resonance (HR-NMR) Spectroscopy. Present study could contribute to determine the date of minimum storage, their optimum conditions, and to properly characterize olive oil.

Keywords: HR-NMR, EVOO, lampant, ageing, soft-deodorization

1 Introduction

The olive oil is deep-rooted in the ancient Mediterranean culture. Nowadays, regulations, expert panellists and advanced technologies are intended to harmonically guarantee the quality and the authenticity of the olive oil.
The present work is as a preliminary study to settle the optimum experimental conditions and data processing for accomplishing the strategies established by the Action Plan for the EU olive oil sector (DG Agriculture & Rural Development, http://ec.europa.eu/agriculture/olive-oil/action-plan_en.pdf, 2012).
The objective of the work was two folded: a) to monitor the evolution of extra virgin olive oil exposed to indirect solar light in transparent glass bottles during four months; b) to identify spectral differences between edible and lampant virgin olive oil by applying high resolution Nuclear Magnetic Resonance (HR-NMR) Spectroscopy.
Present study could contribute to determine the date of minimum storage, their optimum conditions, and to properly characterize olive oil.

2 Materials and methods

A Bruker Avance III instrument operating at the ^{1}H frequency of 700.17 MHz (16.68 Tesla) was used to obtain one-dimensinal HR ^{1}H NMR spectra (HR-NMR). Deuterated chloroform and dimethil sulfoxide were used as dissolvents. The spectral width was set to 18.468 ppm, the time domain data points was 64k, the dwell time was 38.667 μs, and the number of scans

was 216. Spectral pre-processing and data analysis have been carried out by using MestReC and Matlab software.

a) Extra virgin olive oil monitoring evolution

Four extra virgin olive oil (EVOO) cv. Arbequina and Picual from different mills were exposed to indirect sun light in transparent glass bottles along four months. NMR spectra were acquired every month from each sample (from T0 to T4).
One sample of EVOO and one sample of olive oil (OO) were exposed to direct ultraviolet light in a closed chamber at ambient temperature during 24 h. Such treatment involved a forced oil degradation aimed to highlight the most affected regions within the HR-NMR spectra and to facilitate acquisition parameters setting.
HR-NMR spectra were explored in order to detect the presence of relevant peaks that disappear, appear or intensify their height, since such peaks may be used as degradation indicators. Several peaks referred in the reviewed literature along with non referred peaks were identified as potentially useful.
The areas of the relevant spectral regions were computed and then compared by ANOVA in order to reveal significant differences between months. Discriminant analysis was applied as to provide pattern recognition tools.

b) EVOO and non EVOO differences

Six lampant olive oils (LO) cv. Cornicabra and Picual from different mills were compared to four EVOO, two OO samples and two soft deodorised olive oil samples. HR-NMR spectra were explored in order to detect the presence of relevant peaks for discrimination.

3 Results and Discussion

a) Extra virgin olive oil monitoring evolution

Major compounds in EVOO samples, such as oleic, palmitic, linoleic acids…, exposed to indirect sun light in transparent glass bottles were not altered. Thus, relevant peaks identification was focused on minor compounds revealed by forced oxidation with UV, indicated by reviewed references or detected by detailed spectra exploration.
Next figures show the HR-NMR spectrum for each olive oil sample at the starting point of the study (T0) and after UV treatment.

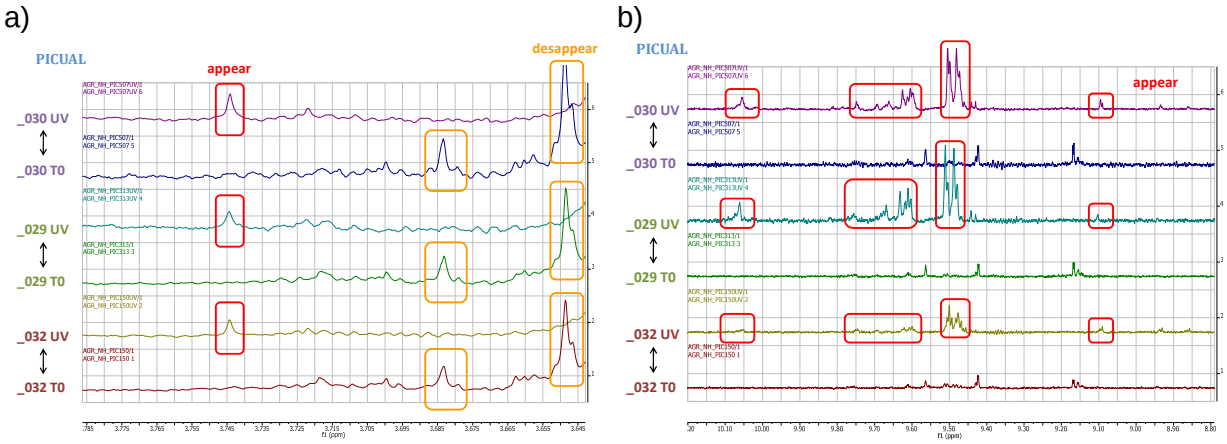
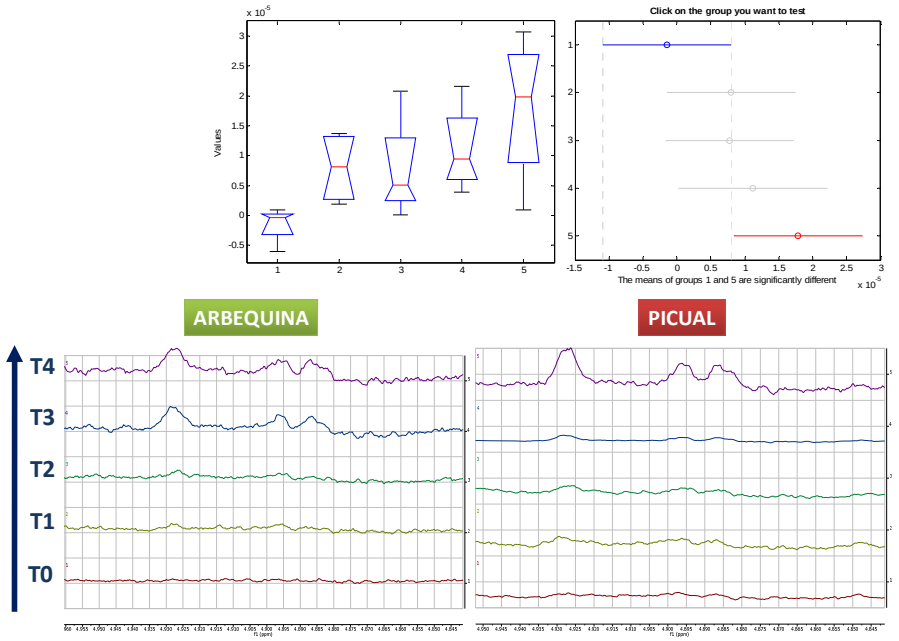


Figure 1. Examples of HR-NMR spectra of cv olive oil cv Arbequina and Picual at T0 and after UV treatment (a: region from 3.645 to 3.785 ppm; b: region from 8.80 to 10.20 ppm). X-axis represents the chemical shift (ppm) and Y-axis the intensity. Framed regions contain the most discriminant peaks.

Four areas of interest were detected when analysing the oil HR-NMR spectra for four months (T1 to T4), which showed an evolution that gave rise to significant differences with T0. Such areas were ascribed to different compounds according to literature (Guillén and Ruiz, 2001; Mannina and Sobolev, 2011; Alonso-Salces et al., 2011; Skiera et al., 2012; Daisa and Hatzakis, 2013; Castejón et al., 2013).

As shown in Figure 2, the area of the spectral regions related to hydroperoxides (Region 1: from 4.80 to 4.90 ppm; Region 2: from 5.45 to 5.75 ppm) increase with time, reaching stabilisation by the fourth (T4) and third (T3) month, respectively, when the area became significantly different from the precedent months; from then onwards the area decreased.

a)



b)

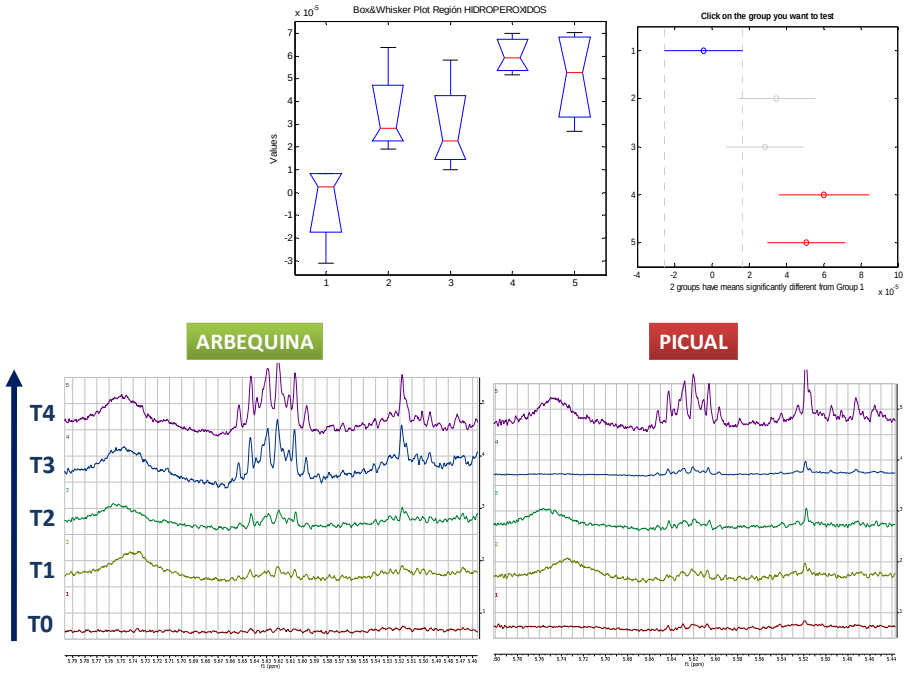


Figure 2. Spectral regions related to hydroperoxides (a: Region 1, from 4.80 to 4.90 ppm; b: Region 2, from 5.45 to 5.75 ppm). First line: left, box and whisker plots of such areas; right: ANOVA results (N=4). Second line, detailed HR-NMR spectra at Region 1 and 2 for cv. Arbequina and Picual.

For aldehydes region (Region 3: from 9.40 to 9.80 ppm) increasing areas were observed with significant differences by the third month of evolution (Figure 3). It is worth noting that the initial area of the spectral region is similar for the four samples analysed, and that the subsequent increase showed different levels, which involved a higher dispersion with the time.

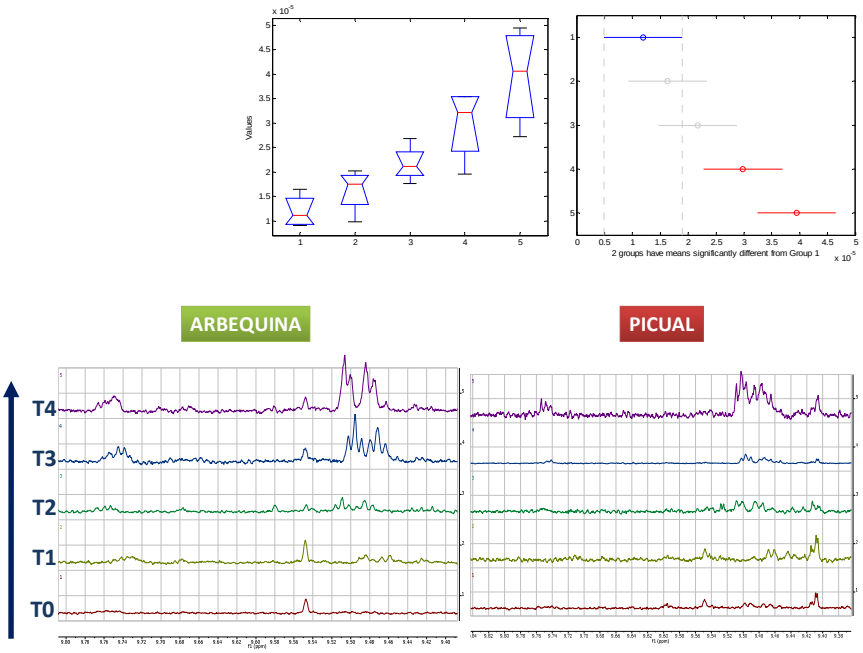


Figure 3. Spectral region related to aldehydes (Region 3: from 9.40 to 9.80 ppm). First line: left, box and whisker plots; right, ANOVA results (N=4). Second line, detailed HR-NMR spectra at Region 3 for cv. Arbequina and Picual.

A decreasing area from double conjugated bonds (Region 4: from 6.50 to 6.59 ppm) was monitored, which stabilised by the second month of evolution (Figure 4). In this case the area dispersion at the beginning of the study is high, which becomes narrower along the following months. Such initial dispersion involved no significant differences between months.

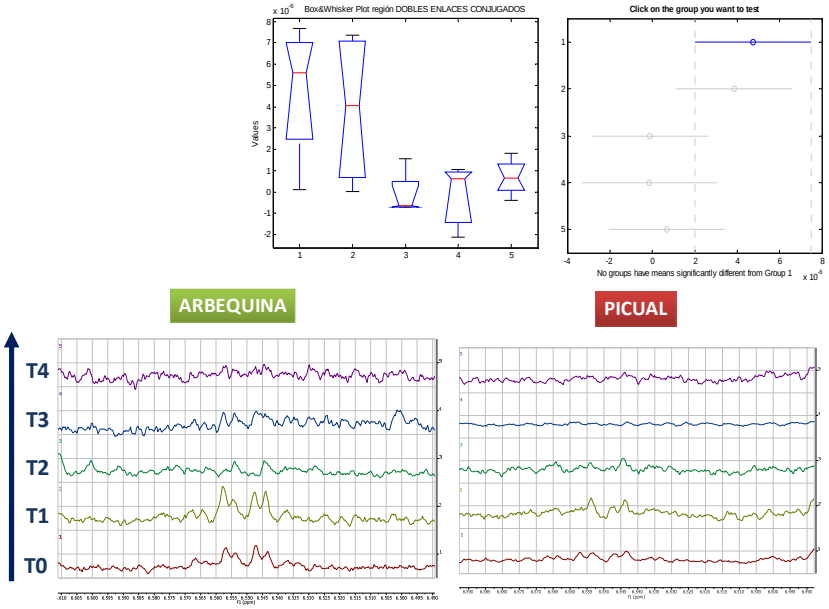


Figure 4. Spectral region related to double conjugated bonds (Region 4: from 6.50 to 6.59 ppm). First line, left, box and whisker plots of such areas; right, ANOVA results (N=4). Second line detailed HR-NMR spectra at Region 4 for cv. Arbequina and Picual.

The relevant spectral regions also included major compounds (from 0.50 to 5.45 ppm), sn-1,2 bonds (from 3.63 to 5.09 ppm) and sn-1,3 bonds (from 2.92 to 4.09 ppm). Differences between samples were found for the evolution of the corresponding areas along the time (Figure 5). Besides, a discriminant analysis depicted an evolving spectral pattern.

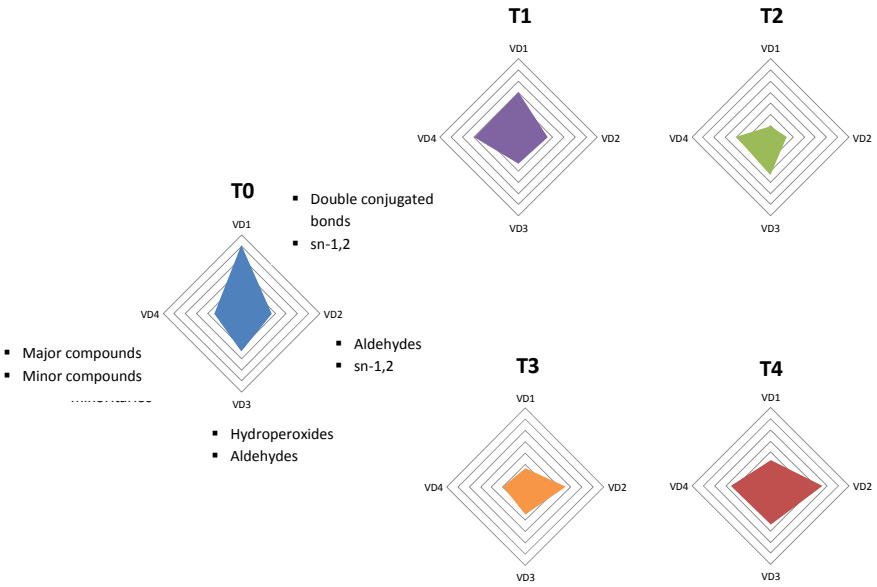


Figure 5. Radial graphs of scores derived from discriminant analysis on the intensity of the spectra at the different regions within HR-NMR spectra.

b) EVOO and non EVOO differences

Several spectral regions including minor peaks were found showing characteristic differences between the analyzed samples, EVOO and non EVOO (Figure 6). In addition, signal arising from oil compounds related to organoleptic characteristics were observed in lampante samples such as “bitter”, “vinegary” and “green-fruity” (Mannina and Sobolev, 2011).

For EVOO two singular peaks were identified at 9.55 ppm and at 9.15 ppm. Distinctive regions related to non EVOO were identified ranging from 9.65 to 9.80 (Region 1), from 9.45 to 9.52 ppm (Region 2), and from 5.47 to 5.67 ppm (Region 3).

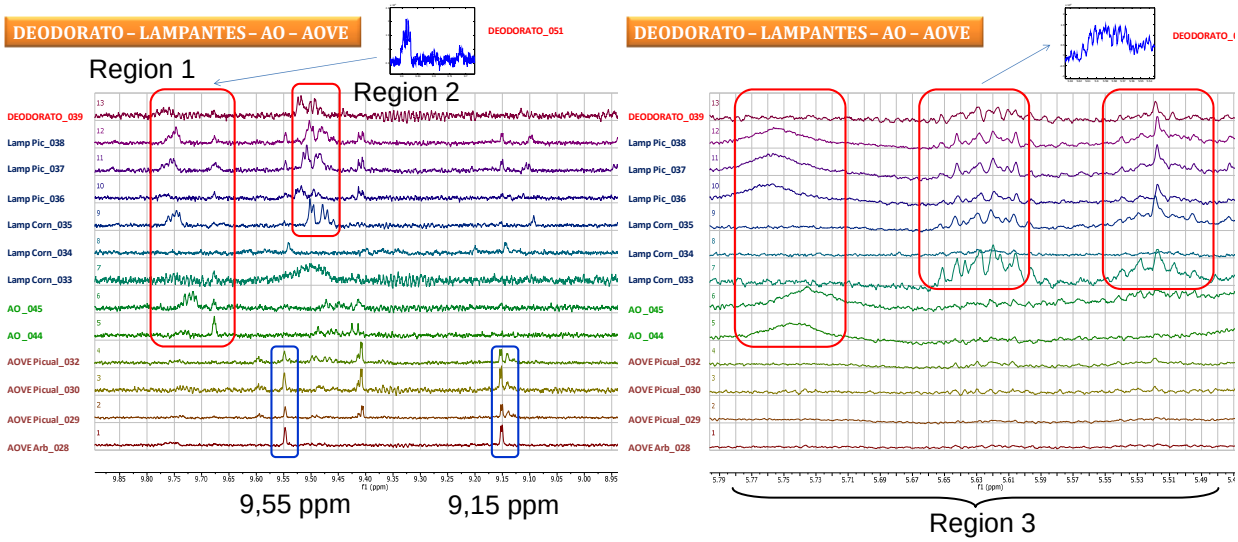


Figure 6. Identification of differentiated regions between deodorates, lampant, olive and extra virgin olive oils. (Region 1, from 9,65 to 9,80 ppm; Region 2, from 9,45 to 9,52 ppm; Region 3, from 5,47 to 5,67 ppm; peak at 9,55 ppm; peak at 9,15 ppm). Peaks framed in blue are characteristic of EVOO; peaks framed in red are characteristic of non EVOO.

In order to identify soft deodorized lampant olive oils is a major requirement to verify that Regions 1 to 3 appear exclusively in deodorates but not in EVOO, and that peaks at 9.55 and 9.15 ppm are uniquely related to EVOO. In such case, different areas ratios could be computed as fraud indicators.

4 Conclusions

HR-NMR spectroscopy is a source of information that provides huge amount of data in a single experiment where most of the components in the sample are detected independently of their different nature. The identification of characteristic spectral regions and the monitor of their evolution are feasible by applying such technique.

Further work is foreseen with larger set of samples and sampling frequency for both, the generation and validation, of reliable and robust models through the application of a variety of chemometric methods such as PCA, PLS-DA, cluster analysis, etc.

5 Acknowledgements

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